PATENT COOPERATION TREATY

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From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
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PTO/PCT Rec'd 08 MAR 2002
41 50 411

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	26.09.2001
Applicant's or agent's file reference: D 2145 PCT/2	**REPLY DUE** **within 2 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/08827	08/09/2000	10/09/1999

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
EPIDAUROS BIOTECHNOLOGIE AG et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☒	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☒	Certain document cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 10/01/2002.

Name and mailing address of the international preliminary examining authority:
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Express Mail Label
No. EV 045436966 US

I. Basis of the opinion

1. With regard to the **elements** of the international application (Replacement *sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"*):

Description, pages:

1-47 as originally filed

Claims, No.:

1-43 as originally filed

Drawings, sheets:

1/7-7/7 as originally filed

Sequence listing part of the description, pages:

1-45, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This opinion has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 12-14,25-29,31-34,42,43(complete);1-11,15-24,30,35-41(partial),

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 12-14,42,43 are

so unclear that no meaningful opinion could be formed (*specify*):
see separate sheet

☒ the claims, or said claims Nos. 12-14,25-29,31,32 are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 33,34(complete);1-32,35-43(partial).

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	9,35,36
Inventive step (IS)	Claims	1-8,10,11,15-24,30,37-41
Industrial applicability (IA)	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item II

Priority

1. Priority was checked with respect to the first and fully searched invention referred to in the present application (see Item V 1.2). Priority was found not to be valid as the priority document does not disclose the molecular variant M20 which shows a nucleotide substitution G-201A.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims relating to inventions in respect of which no International Search Report has been established (claims 33, 34(completely); claims 1-32,35-43(partially)) need not to be the subject of International Preliminary Examination (Rule 66(1)(e) PCT; see PCT/ISA/210). Accordingly, only those parts which are identified in the International Search Report as having been searched are subject of this International Preliminary Examination. With respect to the sequences referred to in the present invention, the sequences SEQ ID NO: 56, 17, 18, 36 and 37 have been searched as these are considered to relate to the first invention (see PCT/ISA/210).

2. Claim 42 refers to the use of a drug or a prodrug comprising "a polynucleotide of any one of claims 1-4 in its genome". The meaning of this claims is so unclear that no meaningful opinion can be formed (Art 34(4)(a)(ii) PCT). The same applies to the dependent claim 43.

3. Claim 12 refers to a molecule which is complementary to a polynucleotide of any of claims 1 to 3. The polynucleotides referred to in claims 2 and 3 are only defined by them coding for a at position -201 mutated form of hPXR. These polynucleotides may, however, encompass further undefined sequences. Therefore, claim 12 lacks clarity and support by the description to such an extent that no meaningful opinion can be formed on said claim (Art 34(4)(a)(ii) PCT). The same arguments also apply to claims 13 and 14.

4. Claim 25 refers to a method of diagnosing in a sample a disorder related to the molecular variant of the hPXR gene. In view of the non-unity objections raised by the ISA only the first invention has been fully searched (see Item V, 1.2). The resulting subject-matter relates to the mutation G-201A which, according to the description of the present application, has no impact on the protein being produced from that molecular variant. In other words, the protein is identical to the wild type form (page 41, Table 4) and thus appears to be unrelated to a disorder. Therefore, claim 25 lacks support by the description to such an extent that no meaningful opinion can be formed (Art 34(4)(a)(ii) PCT). The objection is raised against claims 26-29, 31 and 32.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Basis for the assessment of novelty, inventive step and industrial applicability

1.1 Reference is made to the following document/s/:

D1: WO 99 48915 A (GLAXO GROUP LTD ;KLIEWER STEVEN ANTHONY (US); WILLSON TIMOTHY MARK) 30 September 1999 (1999-09-30)

D2: LEHMANN J M ET AL.,: 'The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions.' JOURNAL OF CLINICAL INVESTIGATION, vol. 102, 1 September 1998 (1998-09-01), page 1016-1023 XP000909297 cited in the application

D3: KLIEWER S A ET AL: 'AN ORPHAN NUCLEAR RECEPTOR ACTIVATED BY PREGNANES DEFINES A NOVEL STEROID SIGNALING PATHWAY' CELL,CELL PRESS, CAMBRIDGE, NA,US, vol. 92, 9 January 1998 (1998-01-09), pages 73-82, XP000918927 ISSN: 0092-8674

D4: DOTZLAW H ET AL.,: 'The human organ receptor PXR messenger RNA is expressed in both normal and neoplastic breast tissue.' CLINICAL CANCER RESEARCH, vol. 5, August 1999 (1999-08), pages 2103-2107, XP000929536

1.2 The International Searching Authority considered that there are 19 inventions claimed in the international patent application and invited the Applicant to pay additional search fees. As no further search fees were paid by the Applicant, the examination of the present patent application will be restricted to the first and fully searched invention encompassing claims 1-43 (all partially; see PCT/ISA/210 for reasoning). The said invention is related to the molecular variant M20 (page 41, Table 4) of hPXR showing a nucleotide substitution at position -201.

2. Novelty

2.1 Claim 1 of the present application appears to be novel (Art 33(2) PCT) as none of the documents cited in the ISR disclose the sequence of SEQ ID NO: 56 or a polynucleotide encoding for hPXR whereby position -201 is an A or mutated in any other way. The dependent claims 2 and 3 and the claims encompassing claim 1 (claims 4-8, 15-19, 40, 41) are thus also novel (Art 33(2) PCT).

2.2 As disclosed in the description of the present application, no effect is predicted to be associated with the mutation at position -201 (page 41, Table 4). Therefore, the protein resulting from this gene appears to be identical to that resulting from the wild type gene.

2.3 Claim 9 refers to a product ("hPXR protein or a fragment thereof") being produced by a novel process. A product, however, is not rendered novel merely by the fact that it is produced by a novel process. Thus, in view of the above (Item V, 2.2), claim 9 lacks novelty (Art 33(2) PCT) as the prior art documents disclose the hPXR protein (D2, page 1018, Fig. 1D; D3, page 74, Fig. 1A; D4, page 2104, left column; page 2105 Fig. 2).

2.2 Claims 10 and 11 appear to be novel (Art 33(2) PCT) as none of documents cited in the ISR disclose an antibody to hPXR or a fragment thereof. Claim 39 is thus also novel (Art 33(2) PCT).

2.3 Claim 20 appears to be novel (Art 33(2) PCT) as none of the documents cited in the ISR disclose a competitive assay as proposed in the said claim to determine an inhibitor of hPXR. The dependent claims 21-24 and the independent claim 30

are thus also novel (Art 33(2) PCT).

2.4 D2 discloses the use of the ligand binding domain of hPXR, which is located at the C-terminus of the said protein and therefore not influenced by the mutation at position -201 for the detection of hPXR mRNA in different tissues (D2, page 1017, left column, "Northern analysis"; page 1018, Fig. 1B; page 1019 Fig. 2). Thus, claims 35 and 36 lack novelty (Art 33(2) PCT). Similar assays are shown in D3 (D3, page 75, Fig. 2A; page 80 "Northern analysis") and D4 (D4, page 2104, "RNA extraction..."; Fig. 1).

2.5 Claim 37 appears to be novel (Art 33(2) PCT) as none of the sequences SEQ ID NO: 56, 17, 18, 36 and 37 are disclosed in the prior art (see also ITEM V, 1.2). Claim 38 is novel for the same reason (Art 33(2) PCT).

2.6 In conclusion, claims 1-8, 10, 11, 15-24, 30, 37-40 and 41 appear to be novel over the prior art (Art 33(2) PCT).

3. Inventive step

3.1 Claim 1 refers to a polynucleotide which encodes a hPXR polypeptide. The polynucleotide referred to in claim 1c and d differs from the closest prior art documents D1 (D1, Fig. 1), D2 (D2, Fig. 1A) and D3 (D3, Fig. 1A) in that at position -201 of the hPXR gene at a single nucleotide difference exists. As disclosed in the description of the present application, the mutation G-201A found in African individuals is not predicted to affect the encoded protein (page 41, Table 4). The technical problem is thus to provide an alternative polynucleotide encoding the same hPXR polypeptide. The solution referred to in claim 1 is to provide a polynucleotide which differs from the prior art in a single nucleotide at position -201 of the hPXR gene. It appears that this solution lacks an inventive step (Art 33(3) PCT) as it is obvious for the skilled person that due to the degeneration of the genetic code, several codons encode the same amino acids.
In conclusion, claim 1 lacks an inventive step (Art 33(3) PCT).

3.2 Claims 2-8, 15-17, 40, 41 appear not to contain features which in combination with the features of the claims to which they refer fulfill the requirements of Art 33(3)

PCT for inventive step.

3.3 Claim 10 refers to an antibody which binds to the protein in claim 9 and is thus encoded by any of the sequences referred to in claim 1. Claim 10 differs from the closest prior art D1-D4 in that it provides an antibody for hPXR. The technical problem is to provide an antibody. For the skilled person it is a routine procedure to develop antibodies to a protein and the required laboratory procedures are standard knowledge. It is also known to the skilled person that instead of the full protein only a small peptide can be used in these procedures. Moreover, it appears, no particular effect is associated with the antibodies referred to in claim 10. Thus, an inventive step can not be acknowledged for claims 10, 11 and 39.

3.4 Claim 18 refers to a method for the detection of an inhibitor capable of modulating the activity of a variant of hPXR. The method involves contacting a variant hPXR with a compound and measuring the activity of downstream mediators CYP3A4 or CYP3A7. Claim 18 differs from the closest prior art document D2 (D2, claim 11) in that the hPXR protein is encoded by a genetic variant which, however, does not influence the amino acid composition of the protein (see Item V, 2.2 above). The technical problem is thus to provide an alternative polynucleotide encoding the same hPXR polypeptide. The solution referred to in claim 18 is to provide a polynucleotide which differs from the prior art in a single nucleotide at position -201 of the hPXR gene. It appears that this solution lacks an inventive step (Art 33(3) PCT) as it is obvious for the skilled person that due to the degeneration of the genetic code, several codons encode the same amino acids.
Claim 19 lacks an inventive step (Art 33(3) PCT) for the same reasons.

3.4 Claim 20 differs from the closest prior art document D2 (D2, claim 11) in that the compound screening method is based on a competitive assay in which the compound to be tested is measured against a known compound which binds to hPXR. The technical problem is to provide an improved method for compound screening. The solution is to employ a competitive assay. It appears that an inventive step (Art 33(3) PCT) can not be acknowledged for said solution as it belongs to the standard knowledge of the skilled person to perform within the search of a suitable, activity modulating compound both, direct inhibition/enhancing studies as in D1 (D1, claim 11) and competitive assays as

proposed in claim 20 of the present application.

3.5 Dependent claims 21-24 and independent claim 30, the latter encompasses the claims 20-24, appear not to contain features which in combination with the features of the claims to which they refer fulfill the requirements of Art 33(3) PCT.

3.6 Claim 37 refers to oligonucleotides of 15-50 nucleotides for detection of a polynucleotide as referred to in claims 1-3 and/or genotyping of hPXR alleles. In view of the unity objection raised by the ISA (see Item V 1.2 above), claim 37 is limited to oligonucleotides which are related to the nucleotide at position -201 of the hPXR gene and thus to the sequences identified in SEQ ID NO: 56, 17, 18, 36 and 37 of the present application. These sequences are not disclosed in the prior art. The sequence of SEQ ID NO:17 is located within exon 1b and can due to its orientation not be used to, for example, determine whether or not the mutation G-201A is present (Fig. 4 "Exon1a&1b"). This sequence thus represent a random selection of an oligonucleotide from a known sequence (D1, SEQ ID NO:13). A technical problem appears not to be associated with the sequence SEQ ID NO:17. Therefore, an inventive step can not be acknowledged for claim 37 (Art 33(3) PCT). Claim 38 lacks an inventive step for the same reason (Art 33(3) PCT).

4. Industrial applicability

4.1 The subject-matter disclosed in the claims 1-8, 10, 11, 15-32, 37, 38-41 of the present application appears to be industrially applicable (Art 33(4) PCT).

Re Item VI
Certain documents cited

1. Even if the Applicant amends the claims so that their priority is valid, the following documents, which have an earlier priority and filing date than the present application, may be of relevance for the examination of the present application in its regional or national phase.

   a: WO 99 48915 A (GLAXO GROUP LTD ;KLIEWER STEVEN ANTHONY

(US); WILLSON TIMOTHY MARK) 30 September 1999 (1999-09-30)

b: EP-A-1 024 193 (CHUGAI PHARMACEUTICAL CO LTD) 2 August 2000 (2000-08-02)

Re Item VII

Certain defects in the international application

1. The expression "herein incorporated by reference" or equivalents thereof (e.g. page 2, first paragraph) in the description of the present application should be deleted (Guidelines, Section IV, II-4.17).

2. To meet the requirements of Art 5 and Rule 5 PCT, the documents D1-D4 should be identified in the description and the relevant background art disclosed therein should be briefly discussed if the subject-matter for which these documents are relevant prior art remains in the claims.

Re Item VIII

Certain observations on the international application

1. Claim 1 lacks clarity (Art 5 and 6 PCT) as the sequence of the hPXR gene is only defined by an accession number to a database but not as a sequence within the application. Further, it should be clarified that the position -201 refers to the wild type sequence to ensure that the nucleotide exchanges can be correctly understood (Art 6 PCT). In addition, there appears to be no basis in the description for a "nucleotide deletion, an additional nucleotide or a nucleotide deletion and a nucleotide exchange" at position -201 of the hPXR gene in the description of the present application (Art 6 PCT) other than the exchange G-201A (page 41, Table 4).

2. The term "variant" in claim 2 lacks clarity and its scope appears to lack support by the description (Art 6 PCT).

3. There appears to be no support by the description (Art 6 PCT) for a "nucleotide

deletion, addition and/or substitution" referred to in claim 3 other than the exchange G-201A (page 41, Table 4). Moreover, claim 3 suggests that the nucleotide exchange results in an "altered expression" of the hPXR gene. However, the mutation G-201A affects the coding of the protein hPXR but not the promoter responsible for the expression of hPXR. Thus, this aspect of claim 3 lacks support by the description (Art 6 PCT).

4. Claim 18 lacks clarity (Art 6 PCT) as the subject-matter of this claim is only defined by the result to be achieved lacking technical features ("... components capable of providing a detectable signal in response to drug metabolisation, with a compound to be screened under conditions...").

5. Claim 20 refers to a method of identifying and obtaining an hPXR inhibitor capable of modulating the activity of a "molecular variant of the hPXR gene" or its "gene product". The method represents a competitive assay on a peptide/protein obtained from the sequences defined in claims 1-3. It is unclear how by assaying compounds on a gene product, an inhibitor can be found which is active on the gene encoding said gene product (Art 6 PCT).

6. The term "derivative" in claim 30 lacks clarity and support by the description (Art 6 PCT).

7. The term "about" in claim 37 results in a lack of clarity. Further, claim 37 appears to be contradictory in itself as, for example, the said oligonucleotide can not be 15 nucleotides long and at the same time comprise the sequence of SEQ ID NO 17, which is already 24 nucleotides long (Art 6 PCT).

8. The term "substance" in claim 39 lacks clarity and support by the description (Art 6 PCT). Further, the phrasing of claim 39 results in a lack of clarity (Art 6 PCT).

9. Claim 21 should be clarified in that the "second complex" replaces but not co-exists with the first complex referred to in claim 20 (Art 6 PCT).

10. In view of the subject-matter disclosed in present application, the number of 22 independent claims appears to be excessive resulting in a lack of clarity (Art 6

PCT) and conciseness of the application (Rule 6.1a PCT).

Concluding remarks

1. The Applicant is invited to file new claims which take account of the above comments and comply with Art 6 and Rule 6 PCT.

2. If the applicant thinks that all objections raised in this communication are overcome, then the description should, at the same time, be brought into conformity with the amended claims. Care should be taken during revision not to add subject-matter which extends beyond the content of the application as originally filed (Art 34(2)(b) PCT). Any statements of problems or advantages should be restricted to the letter of reply.

3. The amendments should be filed by way of replacement pages, avoiding unnecessary recasting of the description. The Applicant should also take account of the requirements of Rules 5 and 6 PCT. In particular, fair printed copies of the amendments should be filed.

4. In the reply, the parts of the application, as originally filed, which form the basis for the amendments (Art 34(2)(b) PCT) should be clearly indicated.

5. In order to expedite the procedure, the Applicant is kindly asked to clearly point out where the amendments have been made, possibly by enclosing a copy of the original pages with the corrections in the manuscript.